Food Safety Inspection Service (FSIS) Technical paper:

Derivation of excess iron limits for meat products produced by Advanced Recovery Systems.

6/21/99

Determining the maximum acceptable level of excess iron in meat products produced by advanced recovery systems

Introduction

As discussed in the FSIS response to comments presented in the preamble, FSIS is using an excess iron measurement for evaluating process control because this measure is associated with bone marrow in the product. The assumption is that there is a significant probability that more than negligible amounts of bone marrow would be present in product with elevated excess iron measurements. If an obtained excess iron measurement is larger than a statistically defined amount, then the obtained measurement is considered elevated.

The objective of the rule is to "provide clear standards ....that include adequate markers for bone-related components (levels consistent with defects anticipated when meat is separated by bone by hand)." FSIS is not saying that excess iron measured levels of inadequately processed meat derived from hand deboning becomes the standard for product produced by advanced recovery systems. Rather, the above objective is interpreted to mean that acceptable excess iron measured levels for product to be labeled as meat would be based on the maximal or worst case expected (or anticipated) excess iron measured levels of meat derived from hand deboning that would be considered as being produced under acceptable manufacturing practices. If hand – deboned product were to have excess iron measured levels greater than these maximal or worst case expected (or anticipated) excess iron measured levels then it would be assumed that the product was not produced under acceptable manufacturing practices. Thus if a product produced by advanced recovery systems has excess iron measured levels greater than these maximal or worst case expected excess iron measured levels, then there is a significant probability that the high iron levels in the product is due to the incorporation of more than negligible bone marrow into the product.

Statistical criteria for determining that specified product (lot) was produced under acceptable manufacturing practice are derived by considering the distribution of an appropriate product characteristic (such as excess iron) when the product is produced under acceptable manufacturing practices and choosing a percentile, p, of this distribution as a demarcation value, D(p). Thus, if, for product produced in a lot, the measured characteristic is greater than D(p), it is assumed that the lot was not produced under acceptable manufacturing practice. The confidence that this is a true assumption and thus a correct decision is greater than p, or, in other words, there is less than a (1-p) probability that the product actually was produced under good manufacturing practice even though the decision was made that it was not so produced. The choice of p is based on an assessment of the relative costs and risks associated with incorrect decisions, and, lacking some compelling reason, is often set between 95% to 99.9%. The choice of 99.9% would correspond to approximately 3 standard deviation units when the distribution is symmetric and normal. Using 3 standard deviation units is a common choice in quality control when there is desire to be highly confident that a decision to reject a product as being produced under good manufacturing practice is a correct decision. For the regulation, since FSIS does not desire to be overly strict and cause unnecessary economic hardship to the industry when there is

not a health concern, a choice of 99.9% confidence or 3 standard deviations units above a specified target is used for determining all tolerances.

To determine the distribution of excess iron measurements in hand-deboned meat product, the measurement error due to repeatability will be accounted for. Information from USDA's Agriculture Research Service (ARS) is used to establish a repeatability standard deviation of 0.16 mg/100g for a single iron determination. The data and the results of statistical analysis of the data are presented as an attachment to this report. The repeatability standard deviation of protein is set equal to $0.03x^{0.64}$ where x is the % protein content obtained using the Kjeldahl procedure with mercury catalyst, based on results from a paper (Price, Cindy G.; Webb, Neil B.; Smith, Wertice J.; Marks, Harry M.; Yoffe Aron M. 1994, "Comparison of Mercury and Copper based catalysts in the Kjeldahl determination of nitrogen in meat and meat products: collaborative study", J. of AOAC International, vol. 77, 6: p. 1542-1556).

Maximum mean level (MML) for a lot

The term "lot" in this setting is used to represent product produced by advanced recovery systems that has been processed uniformly. It is assumed that the starting materials used, the calibrations of the machinery, and other processing parameters that affect the composition of the product would be as uniform as possible. Thus, a lot does not necessarily represent the product produced in a day. Within a lot, the excess iron measurements for different samples of the product would be different due to unavoidable differences in ratios of iron to protein in different animals and analytical variations in the measured iron and protein levels in samples. However, the lot would have a mean excess iron level, which would reflect processing control and would provide, therefore, an appropriate measure for evaluation. In accordance with the above objective of determining the excess iron measured limits of product produced by advanced recovery systems that can not be labeled meat, the first step is to determine the maximum mean level, MML, of excess iron for a lot. From the discussion in the previous section, the MML is equal to 3 times the "between lot" standard deviation of the excess iron for meat derived by hand deboning. Once this level is determined, then compliance criteria, based on chemical analysis of samples, are developed which take into consideration the between sample and analytical measurement variability. In particular, the criterion for an individual sample, based on duplicate analyses (for both protein and iron) is derived.

In order to derive the excess - iron MML for a "lot" and a criterion for an individual sample, the 1996 FSIS AMR neckbone survey results for the meat derived from hand deboning will be used. From each of two establishments, 27 samples of meat derived from hand deboning were collected on various days of production with 3 samples a day (Table 1). The FSIS procedure to measure iron employed a hydraulic wet acid digestion procedure. However, another method, performed by ARS scientists, which uses a dry ash procedure for digestion, obtained iron results approximately double those originally obtained by FSIS. Furthermore, the results obtained by the ARS dry ash procedure were more consistent with levels reported in the HNS Handbook 8 levels for hand-deboned meat. Consequently, the excess iron values will be calculated using iron results obtained by the ARS dry ash procedure. For samples for which there were not ARS dry - ash procedure results, the FSIS results were multiplied by 2.12 (which was the average ratio of the dry - ash procedure results to the FSIS results). For the 54 samples of hand–deboned product, 45 of them were

analyzed by the ARS dry – ash procedure. Table 2 provides a comparison of all the FSIS and ARS obtained results provided to FSIS by ARS.

In actuality, the meat derived from hand deboning in the survey might have been heterogeneous, so that within a day there might be more than one "lot" of homogeneous product. In an analysis of variance, the day within an establishment effect had a significance level (p- value) of 0.15 (based on 16 degrees of freedom). An examination of the data did not reveal any particular result or set of results that could be classified as an outlier. This suggests that the between day variability compared to the within day variability was not relatively large and that a single day might consist of more than one lot. Thus, it would be expected that the actual between lot variance might be larger than the measured between day variance. Since the between sample (within day) variance is considerably larger than the repeatability variance, it is possible that a sample "represents" a lot. The "truth" may actually be between the two extremes, identified here, of a day representing a lot or a sample representing a lot. Thus a "compromise" calculation is used for determining the between lot variance component. Specifically, the between lot variance component is set equal to "p" percent of the within day variance component plus the between day variance component, and the within lot variance is set equal to 1-p%/100 of the measured within day variance component. For the regulatory derived criteria, p was set equal to 50%, so that the between lot variance is assumed to equal the sum of the between day variance plus ½ of the between sample/within day variance, and the within lot variance is assumed to be equal to ½ the between sample/within day variance.

Excess iron for hand–deboned product, ExFe, is computed as iron minus 0.138 times the percentage protein (Fe –0.138protein). The factor 0.138 is the ratio of the average iron to average protein of the hand deboned neckbone product from the FSIS survey, so that, for this product, the mean of the excess iron results is 0.00 mg/100g. This factor is also equal to the average iron to protein ratios of the samples. As stated above, the repeatability standard deviation for the iron measurements is assumed to be equal to 0.16 mg/100g, which was derived from information obtained from ARS (see attachment), and the repeatability of protein measurements is equal to $0.03x^{0.64}$ where x is the percent protein in the sample (REF). Using these values, from the formula for excess iron, ExFe = Fe - 0.138x, the average repeatability variance from the hand – deboned samples was calculated to be equal to 0.0265. An analysis of variance (AOV) of the sample excess iron results is presented in Table 3.

The between day/establishment variance, from Table 3, is estimated to be 0.13408, and the between sample/within day variance, after accounting for the measurement variance is estimated to be 0.2875. Thus, the between lot variance, assuming p= ½, is 0.13408 + ½ 0.2875 = 0.2778, so that between lot standard deviation is 0.5271 mg/100g. The maximum mean excess iron for a lot (MML) is 3 times the between lot standard deviation = 3(0.5271) = 1.5813 mg/100g.

Determining Tolerance for Compliance Purposes

FSIS may take samples to evaluate whether or not establishments are producing product produced by advanced recovery systems with "lot" averages greater than the MML, 1.5813 mg/100g. The amount of product in a sample is assumed the same as the sample amounts of the 1996 FSIS survey of product derived from advanced recovery systems. Of course the product produced by advanced recovery systems is produced differently than the

corresponding meat derived by hand deboning, and thus the within "lot" variances could be different. The within day variance of excess iron results for the product derived from advanced recovery systems was computed, by an AOV, to be 0.5922, so that the within day standard deviation is equal to 0.7695 mg/100g, which is larger than the within day standard deviation of the hand–deboned product, 0.5603 mg/100g, given in Table 3. This could mean that the product produced by advanced recovery systems was not produced uniformly throughout the day. In addition, the mean adjusted excess iron measurement for product derived from advanced recovery systems is 3.235 mg/100g (incorporating the 10% adjustment as described in the response to comments given in the preamble to this rule), which is larger than the maximum acceptable mean excess iron for a lot, MML, of 1.5813 mg/100g, derived above, assuming p=50%, or even the maximum estimate of MML equal 1.95 mg/100g obtained when assuming p=100% (each sample from the survey represents a lot). Hence, the product produced by advanced recovery systems of the FSIS survey would not be considered comparable to meat derived by hand deboning, thus can not, justifiably, be used for determining the within lot standard deviation for product produced by advanced recovery systems that is comparable to meat. It might be that, under good manufacturing practices, product produced by advanced recovery systems that was produced uniformly would be more homogeneous than its counterpart meat derived from hand-deboning, so that the variance would be smaller.

A general sampling plan is to take n samples throughout the lot, composite them, and perform n_r analytical measurements. If Fe_i and pr_i represent the i^{th} iron and protein results, respectively, then the adjusted excess iron, aExFe, result for a n – sample composite is

$$aExFe = \sum Fe_i / n_r - (0.138)(1.10)\sum pr_i / n_r$$

The expected variance of the adjusted excess iron estimator, aExFe, for n – sample composites obtain by such a sampling plan is:

$$\text{var}(aExFe) = \sigma_s^2 / n + \sigma_r^2 / n_r$$

where σ^2_r is the repeatability variance of adjusted excess iron measurements and σ^2_s is the between sample/within lot variance of the adjusted excess iron.

In order to select a specific sampling plan (that is, the number of samples for a lot) producer and consumer risks (probabilities of the lot passing the test) must be selected. The MML represents the maximum mean level for a lot that does not result in a non-compliance determination, thus if a lot had a mean equal to the MML then there should be a high probability that this lot would not fail and pass the sampling plan. As discussed above, for determining tolerances, FSIS is selecting 3 standard deviations above the mean, so that if a result on a n – sample composite is obtained that exceeds the demarcation value, then there would be approximately 99.9% confidence that the mean for the "lot" exceeds the MML, and thus the product within the lot would not be considered comparable to meat. To compute a consumer risk, the probabilities of passing a lot with mean excess iron level equal to 6 standard deviations above zero are determined.

FSIS laboratories would analyze a compliance sample at least in duplicate (see attachment). Thus it is assumed that n – sample composites are analyzed in duplicate, so that $n_r = 2$. Because of the factor 1.10, the variance components for this estimator will be different from those given in Table 3. The analysis of variance for the meat derived from

hand deboning was repeated using the above formula. Presented in Table 4, are the derived variance components for the above estimator of the adjusted excess iron statistic for the hand–deboned product.

The protein values do not affect by much the standard deviation, so that it can be assumed that the repeatability variance is 0.0267. The between sample/within lot variance, σ_s^2, as discussed above, is equal to (1-p) times the between sample/within day, where p = ½. Thus , σ_s^2 = ½ 0.2905 = 0.1453, and the expected variance for the adjusted excess iron results for n – sample composites is therefore, Var(aExFe) = 0.1453/n + 0.0267/2. The square root of this quantity is the expected standard deviation.

Table 5 provides demarcation values for determining that a lot has mean excess iron greater than the MML, 1.5813 mg/100g, for different numbers of samples taken from the lot, assuming duplicate measurements on the composite of the samples. An individual sample is when n=1 so that the individual sample limit that is specified in the regulation is 2.776 mg/100g. For purposes of the regulation (for recalling the demarcation value), this is adjusted to 2.800 mg/100g, so that if an obtained sample result (based on the average of duplicate analyses of iron and protein) is greater than or equal to 2.800 mg/100g then the product produced by advanced recovery systems can not be labeled meat (see conclusion section, below).

If a different percentage, p, than 50% of the within day variance component is added to the between day variance component, then different answers are obtained for the individual sample demarcation value and for MML. Figure 1 is a plot of the MML and the individual sample limit The percentage that gives the maximum individual sample demarcation value is 30% and the maximum value is 2.8048 mg/ 100g, with a MML equal to 1.400 mg/100g. The minimum possible derived individual sample limit, obtained when p=100%, is 2.2942 mg/ 100g, with the maximum possible derived MML equal to 1.948 mg/100g.

FSIS Survey of product produced by advanced recovery systems.

Presented in Table 6 are the establishment means of adjusted excess iron for product produced by advanced recovery systems, after the 10% adjustment, aExFe = Fe – (0.138)(1.10)(protein), and the percentage of samples that are greater than or equal to the derived individual sample limit, 2.800 mg/100g. All the establishment means are greater than the MML of 1.5813 mg/100g. Also included in Table 6 is the establishment means of excess iron(not adjusted) of the meat derived from hand deboning. The highest individual excess iron sample result for the hand-deboned meat was 1.76 mg/100g. For product produced by advanced recovery systems, 62% of the samples had adjusted excess iron results that were greater than or equal to 2.800 mg/100g.

Conclusion:

If a mean of results from duplicate analyses on a sample is greater than or equal to 2.800 mg/100g then it is assumed that there is product that is not meat based on the

incorporation of bone marrow. The question of the product (the lot) to which this conclusion applies needs to be answered. In answering, it is assumed that contiguous product is in the same lot. Since an establishment is required to have documentation that its production process is in control, it is assumed that a non-compliant finding is a result of a failure or a deficiency in the process control. A consequence is that all product that is produced before or after the non-compliant sample might also have been produced when the process was not in control and thus could not be labeled. One way of showing that product is not from the same "lot" is to examine the records of values of processing parameters that affect the composition of the product produced by advanced recovery systems or other analytical results from samples of product produced in different parts of the day or on different days and to determine if there are reasons to identify different "lots" and reasons that the non-tested lots would not have non- complying product (mean levels of excess iron less than 1.58 mg/100g).

Figure 1: Plot of derived maximum mean excess iron for lot (MML) and individual – sample excess iron limit as function of percentage, where within lot variance equals the sum of the between day variance plus given percentage of within day variance and within lot variance equals 100-percentage of within day variance.

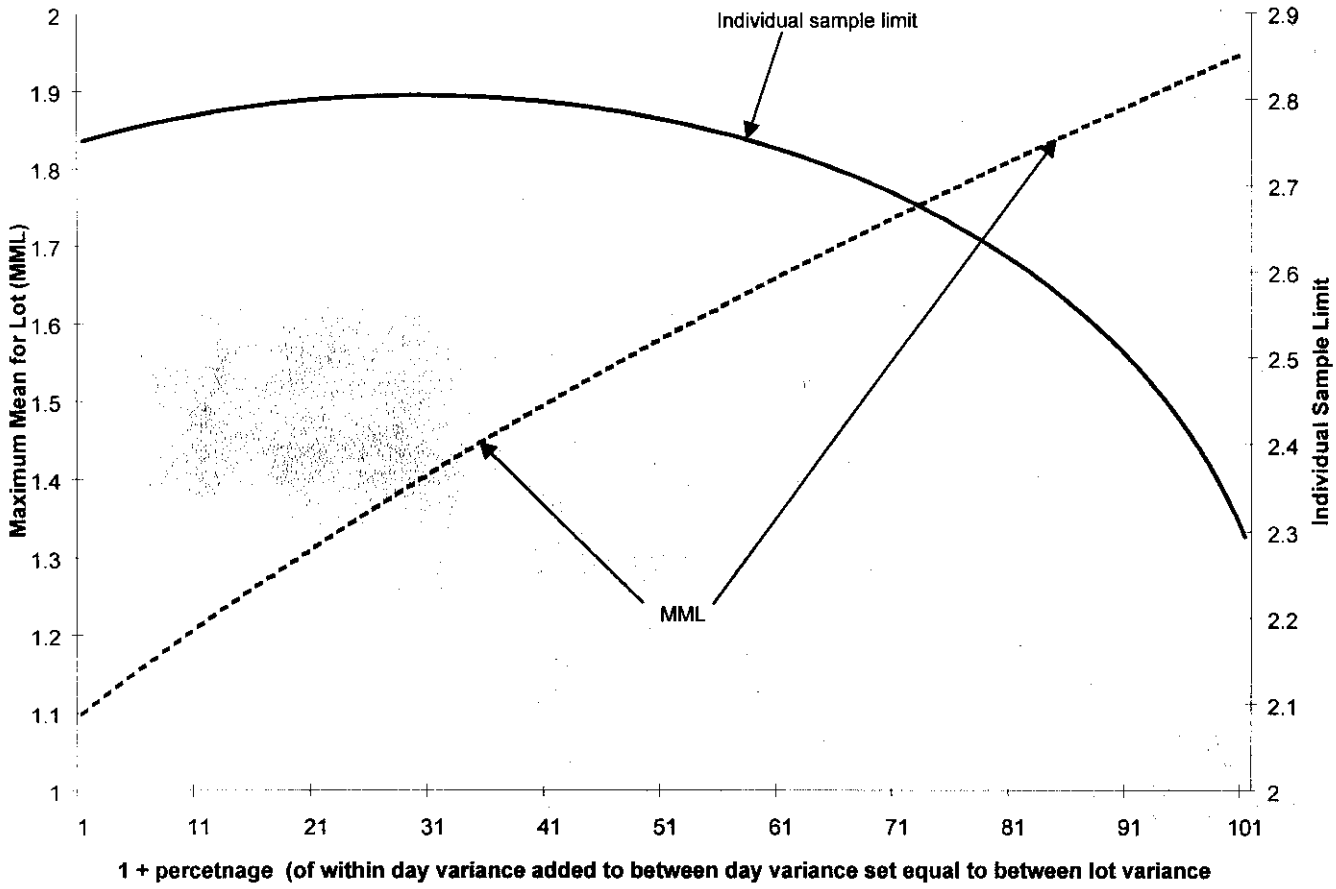

Table 1: Iron and Protein measurements obtained for hand deboned product. *Obtained from 1996 FSIS survey.*

OBS	Est. code	Date of sampling code	Iron dry-ash mg/100g	Protein(%)	Excess iron measure mg/100g
1	5	1	2.26	19.13	-0.38
2	5	1	2.36	19.59	-0.34
3	5	1	2.46	19.32	-0.21
4	5	2	2.74	19.56	0.04
5	5	2	2.60	20.24	-0.19
6	5	2	3.29	19.61	0.59
7	5	3	2.54	21.90	-0.48
8	5	3	2.47	21.38	-0.48
9	5	3	4.01	22.65	0.88
10	5	4	2.50	21.25	-0.43
11	5	4	2.62	21.81	-0.39
12	5	4	2.47	22.72	-0.66
13	5	5	3.04	19.91	0.29
14	5	5	3.22	19.50	0.53
15	5	5	3.79	19.33	1.13
16	5	6	3.22	21.76	0.22
17	5	6	3.44	22.15	0.39
18	5	6	4.14	20.85	1.26
19	5	7	3.22	23.05	0.04
20	5	7	2.99	23.35	-0.23
21	5	7	4.33	21.96	1.30
22	5	8	3.03	23.30	-0.19
23	5	8	2.92	22.95	-0.25
24	5	8	4.55	23.25	1.34
25	5	9	3.18	23.25	-0.03
26	5	9	4.93	23.00	1.76
27	5	9	3.52	22.00	0.48
28	8	1	2.70	20.88	-0.19
29	8	1	2.64	21.54	-0.33
30	8	1	2.43	21.49	-0.54
31	8	2	2.88	21.86	-0.14
32	8	2	2.84	21.07	-0.07
33	8	2	2.88	22.34	-0.21
34	8	3	2.58	21.56	-0.40
35	8	3	2.73	21.91	-0.29
36	8	3	2.62	21.02	-0.28
37	8	4	2.56	21.64	-0.42
38	8	4	2.72	22.92	-0.45
39	8	4	2.48	22.14	-0.57
40	8	5	3.01	22.80	-0.14
41	8	5	3.71	21.09	0.80

Table 1 (cont): Iron and Protein measurements obtained for hand deboned product.

OBS	Est. code	Date of sampling code	Iron dry-ash mg/100g	Protein(%)	Excess iron measure mg/100g
42	8	5	3.35	21.44	0.39
43	8	6	2.45	22.02	-0.59
44	8	6	2.14	20.75	-0.72
45	8	6	1.67	21.79	-1.34
46	8	7	2.98	20.90	0.09
47	8	7	2.31	23.91	-0.99
48	8	7	3.66	22.10	0.61
49	8	8	3.35	22.90	0.19
50	8	8	3.23	23.20	0.03
51	8	8	3.40	22.61	0.28
52	8	9	2.37	20.42	-0.45
53	8	9	1.94	21.84	-1.07
54	8	9	3.82	21.91	0.80

Table 2: Comparison of ARS Dry Ash and FSIS Wet Acid digestion results
Units mg/100g (AMR= product from advanced recovery systems

OBS	Type of Product	FSIS Wet Acid	ARS Dry Ash	Ratio Dry Ash to Wet Acid
1	AMR	2.36	5.08	2.15
2	AMR	3.19	5.90	1.85
3	AMR	2.46	7.03	2.86
4	AMR	1.83	4.13	2.26
5	AMR	2.69	4.13	1.54
6	AMR	2.81	5.15	1.83
7	AMR	2.49	4.80	1.93
8	AMR	1.79	5.18	2.89
9	AMR	2.23	5.59	2.51
10	AMR	2.41	5.97	2.48
11	AMR	7.91	8.32	1.05
12	AMR	4.88	7.02	1.44
13	AMR	2.39	5.56	2.33
14	AMR	2.94	5.23	1.78
15	AMR	2.57	4.99	1.94
16	AMR	2.64	5.08	1.92
17	AMR	2.72	4.97	1.83
18	AMR	2.81	4.88	1.74
19	AMR	2.82	5.09	1.80
20	AMR	2.04	5.26	2.58
21	AMR	3.59	5.36	1.49
22	AMR	3.56	5.19	1.46
23	AMR	2.90	6.17	2.13
24	AMR	3.03	5.43	1.79
25	AMR	2.52	3.53	1.40
26	AMR	3.51	5.61	1.60
27	AMR	3.26	5.33	1.63
28	AMR	3.32	5.03	1.52
29	AMR	2.68	4.76	1.78
30	AMR	3.17	5.42	1.71
31	AMR	3.90	6.05	1.55
32	AMR	2.31	5.00	2.16
33	AMR	2.70	6.43	2.38
34	AMR	1.51	4.93	3.26
35	AMR	2.36	5.37	2.28
36	AMR	2.26	5.07	2.24
37	AMR	1.79	5.26	2.94
38	AMR	1.70	4.44	2.61
39	AMR	2.42	4.37	1.81
40	AMR	1.70	4.89	2.88
41	AMR	2.30	6.20	2.70
42	AMR	2.51	6.31	2.51
43	AMR	2.52	5.61	2.23
44	AMR	2.66	6.17	2.32
45	AMR	3.05	4.72	1.55
46	AMR	3.21	6.11	1.90
47	AMR	2.39	6.10	2.55

Table 2 (cont): Comparison of ARS Dry Ash and FSIS Wet Acid digestion results
Units mg/100g (AMR= product from advanced recovery systems

OBS	Type of Product	FSIS Wet Acid	ARS Dry Ash	Ratio Dry Ash to Wet Acid
48	AMR	2.20	4.99	2.27
49	AMR	1.78	6.11	3.43
50	AMR	2.20	5.85	2.66
51	AMR	2.42	5.91	2.44
52	AMR	2.55	6.38	2.50
53	AMR	3.87	6.78	1.75
54	AMR	1.78	4.86	2.73
55	AMR	1.79	6.74	3.77
56	AMR	2.27	5.95	2.62
57	AMR	4.11	6.43	1.56
58	AMR	2.96	6.19	2.09
59	AMR	2.31	4.99	2.16
60	AMR	2.37	4.72	1.99
61	AMR	2.29	4.67	2.04
62	AMR	1.94	5.42	2.79
63	AMR	1.52	5.43	3.57
64	AMR	2.07	5.62	2.71
65	AMR	3.03	5.63	1.86
66	AMR	2.05	7.47	3.64
67	AMR	2.36	6.58	2.79
68	AMR	3.00	5.30	1.77
69	AMR	2.89	4.85	1.68
70	AMR	3.21	4.36	1.36
71	AMR	2.84	5.44	1.92
72	AMR	2.69	5.89	2.19
73	AMR	2.95	6.09	2.06
74	AMR	3.34	6.33	1.90
75	AMR	3.95	5.91	1.50
76	AMR	4.44	7.52	1.69
77	AMR	3.45	5.37	1.56
78	AMR	3.73	5.47	1.47
79	AMR	3.69	5.94	1.61
80	AMR	2.75	4.89	1.78
81	AMR	2.51	5.69	2.27
82	AMR	2.62	5.65	2.16
83	AMR	2.69	5.93	2.20
84	AMR	2.36	5.07	2.15
85	AMR	1.97	4.42	2.24
86	AMR	2.02	5.40	2.67
87	AMR	2.25	5.91	2.63
88	AMR	3.63	8.95	2.47
89	AMR	3.85	6.98	1.81
90	AMR	4.08	7.13	1.75
91	AMR	2.57	5.70	2.22
92	AMR	3.03	8.19	2.70
93	AMR	1.88	3.26	1.73
94	AMR	2.64	6.52	2.47

Table 2(cont): Comparison of ARS Dry Ash and FSIS Wet Acid digestion results
Units mg/100g (AMR= product from advanced recovery systems

OBS	Type of Product	FSIS Wet Acid	ARS Dry Ash	Ratio Dry Ash to Wet Acid
95	AMR	2.54	7.27	2.86
96	AMR	3.86	7.01	1.82
97	AMR	3.10	6.39	2.06
98	AMR	3.77	5.71	1.51
99	AMR	3.02	6.80	2.25
100	AMR	2.35	5.16	2.20
101	AMR	2.58	4.86	1.88
102	AMR	2.29	5.02	2.19
103	AMR	2.96	3.87	1.31
104	AMR	3.21	5.91	1.84
105	AMR	1.88	5.93	3.15
106	AMR	2.04	5.48	2.69
107	AMR	2.40	4.14	1.73
108	AMR	2.36	5.11	2.17
109	AMR	3.10	4.99	1.61
110	AMR	3.42	5.83	1.70
111	AMR	2.34	4.86	2.08
112	AMR	3.89	4.91	1.26
113	AMR	2.96	6.05	2.04
114	AMR	3.50	6.43	1.84
115	AMR	3.37	5.38	1.60
116	AMR	1.97	4.96	2.52
117	AMR	3.15	5.60	1.78
118	AMR	3.00	6.15	2.05
119	AMR	3.24	5.96	1.84
120	AMR	2.72	5.68	2.09
121	AMR	3.54	6.00	1.69
122	AMR	3.70	6.55	1.77
123	AMR	2.63	4.83	1.84
124	AMR	3.12	5.95	1.91
125	AMR	4.14	8.21	1.98
126	AMR	1.78	4.37	2.46
127	AMR	1.96	5.27	2.69
128	AMR	1.91	3.98	2.08
129	AMR	2.22	5.84	2.63
130	AMR	2.85	5.35	1.88
131	AMR	2.35	6.13	2.61
132	AMR	3.64	7.67	2.11
133	AMR	3.19	7.08	2.22
134	AMR	2.27	6.56	2.89
135	AMR	2.96	5.16	1.74
136	AMR	2.57	6.16	2.40
137	AMR	2.25	6.00	2.67
138	AMR	2.14	6.37	2.98
139	AMR	2.39	6.61	2.77
140	AMR	2.81	6.66	2.37
141	AMR	5.30	6.34	1.20

Table 2(cont): Comparison of ARS Dry Ash and FSIS Wet Acid digestion results
Units mg/100g (AMR= product from advanced recovery systems

OBS	Type of Product	FSIS Wet Acid	ARS Dry Ash	Ratio Dry Ash to Wet Acid
142	AMR	5.13	6.98	1.36
143	AMR	4.36	5.13	1.18
144	Hand	1.06	2.26	2.13
145	Hand	1.10	2.64	2.40
146	Hand	1.09	2.36	2.17
147	Hand	1.06	2.46	2.32
148	Hand	1.34	2.43	1.81
149	Hand	1.40	2.70	1.93
150	Hand	1.78	3.29	1.85
151	Hand	1.56	2.60	1.67
152	Hand	1.82	2.74	1.50
153	Hand	1.78	2.88	1.62
154	Hand	1.90	2.88	1.51
155	Hand	1.79	2.84	1.58
156	Hand	1.43	2.48	1.74
157	Hand	1.56	2.62	1.68
158	Hand	1.65	2.72	1.65
159	Hand	1.48	2.50	1.69
160	Hand	1.37	2.56	1.87
161	Hand	1.60	2.62	1.64
162	Hand	1.18	2.47	2.09
163	Hand	1.26	2.58	2.05
164	Hand	1.69	1.67	0.99
165	Hand	1.53	2.45	1.60
166	Hand	1.21	2.14	1.77
167	Hand	1.74	3.04	1.75
168	Hand	1.70	3.82	2.25
169	Hand	1.07	1.94	1.81
170	Hand	1.51	2.37	1.57
171	Hand	1.01	3.01	2.98
172	Hand	1.25	2.47	1.98
173	Hand	1.20	4.01	3.34
174	Hand	1.14	2.54	2.23
175	Hand	1.41	3.22	2.28
176	Hand	1.77	4.14	2.34
177	Hand	1.64	3.44	2.10
178	Hand	1.14	2.98	2.61
179	Hand	1.58	3.66	2.31
180	Hand	1.62	2.31	1.43
181	Hand	1.19	3.23	2.71
182	Hand	1.10	3.40	3.09
183	Hand	1.19	3.35	2.82
184	Hand	1.22	3.03	2.48
185	Hand	1.60	4.55	2.84
186	Hand	1.44	2.92	2.03
187	Hand	1.46	4.93	3.38
188	Hand	1.40	4.33	3.09

Table 3: Analysis of variance of excess iron results, ExFe, from 1996 FSIS survey of hand-deboned neckbone samples, (2 establishments, 27 observations per establishment). ExFe = iron – 0.138protein.

Source of Variation	Variance	Standard deviation
Between establishment	0.08054	0.2838
Between Day within establishment	0.05354	0.2314
Sum: Between establishment/day	0.13408	0.3662
Within day including measurement error	0.3140	0.5603
Measurement error	0.0265	0.1628
Between sample/Within day	0.2875	0.5362
Total variance	0.4481	0.6694

Table 4: Analysis of variance of adjusted excess iron, aExFe, results from 1996 FSIS survey of meat derived from hand deboning. aExFe = iron – (0.138)(1.10)protein.

Source of Variation	Variance	Standard Deviation
Between establishment/day	0.1333	0.3651
Within day including measurement error	0.3172	0.5632
error	0.0267	0.1633
Between sample/Within day	0.2905	0.5390

Table 5: Limits for determining that a lot has mean adjusted excess iron, aExFe, greater than 1.58mg/100g for n – sample composites as function of the number of samples, n, per lot, assuming duplicate analysis on the composite of the samples. The aExFe n – sample composite result is equal to the mean of the iron results minus the product of 0.138, 1.10 and the mean protein result. The derived limit is equal to 3 expected standard deviations above the maximum mean for a lot (MML) = 1.5813 mg/100g. Also presented are the probabilities of passing a lot with a true excess iron mean = 3.1626 mg/100g (= 6 between lot standard deviations above zero excess iron).

Number of Samples	Limit	Prob. (%) passing lot mean=3.163
1	2.776	16.5821
2	2.461	0.8345
3	2.327	0.0385
4	2.250	0.0021
5	2.199	0.0001

Table 6: Summary of excess iron results from 1996 FSIS neckbone survey.
The hand–deboned excess iron results are computed as: iron –0.138protein,
The product produced by advanced recovery systems (AMRS) excess iron results are computed as: iron – (0.138)(1.10)protein.

establish-ment code	number of samples	mean excess iron	percent samples > 2.776
8 hand	27	0.221	0.00
9 hand	27	-0.221	0.00
all hand	54	0.000	0.00
1[a] AMRS	27	2.778	40.74
2 AMRS	24	4.950	87.50
3 AMRS	16	3.280	56.25
4 AMRS	27	2.656	33.33
5 AMRS	25	3.443	76.00
6[b] AMRS	25	3.560	84.00
7 AMRS	19	3.065	57.90
ALL AMRS	163	3.235	61.96

[a]) establishment used Protecon machine, while others used Hydrosep machines.
[b]) establishment did not perform desinewing operation.

Attachment:

Repeatability of iron measurements using the ARS Dry-Ash procedure

Data to determine the repeatability of the ARS Dry- Ash procedure was provided to FSIS by Dr. Bob Windham of ARS. Analyses were conducted on beef samples. For further details contact Dr. Bob Windham. The first data set consists of duplicate results obtained by the same laboratory on 47 samples. The second data set are results from a 3-laboratory, 5-sample collaborative study, where each sample was analyzed in duplicate by each lab.

The results from the 47 samples of the first data set are given in Table 1. Statistical analysis did not indicate a non-zero correlation of the standard deviations and mean levels of the samples, so that it is assumed that the repeatability standard deviation does not depend upon the level of iron in the sample. Figure 1 is a plot of the sample standard deviations versus the sample means for the 47 samples. The line represents a quadratic fit. It can be seen from this graph the 5 data points that have standard deviations greater than 0.5 mg/100g. The standard deviations of these 5 data points can be assumed to be outlier standard deviations. This can be seen by computing the ratio of the maximum sample variance to the sum of the sample variances and comparing this ratio to appropriate percentiles of a beta distribution (Hawkins, D. M., 1980, Identification of Outliers, Chapman and Hall, New York, NY, Appendix 9). Specifically, let $v_{(j)}$ be a random variable representing the j^{th} ordered sample variance from k samples. The ratio of the maximum sample variance to the sum of the sample variances,

$$r_k = v_{(k)} / \sum_{j=1}^{k} v_{(j)}$$

is compared to an appropriate percentile of a beta distribution with parameters ½ and (k-1)/2. To determine whether $v_{(k)}$ is an outlier with respect to the set $\{v_{(j)}$, for $j<k\}$, the observed value of the ratio, r_k, is compared to the $1-\alpha/k$ percentile of the beta distribution, where α represents the significance of the statistical test of $v_{(k)}$ being an outlier. For k=43, ... 47, the ratios r_k were computed and the corresponding significance levels, α_k, were determined. For k=47, $\alpha_{47} = 0.00029$, so that the highest computed variance can be considered as an outlier. For k=46, $\alpha_{46} = 0.00007$, so that the second highest variance can be considered as an outlier. Also, $\alpha_{45} = 0.00482$, $\alpha_{44} = 0.03097$ and $\alpha_{43} = 0.03577$, so that the five highest variances can be considered as outliers. Assuming that the variances on these five samples are outlier results and thus excluding them from the analysis, the repeatability standard deviation from the remaining 42 samples is estimated (by computing the square root of the mean of sample variances) to be 0.161 mg/100g. If the sample with standard deviation 0.58 mg/100g is included in the calculations, then the estimated repeatability standard deviation is estimated to be 0.182 mg/100g. Further, the distribution of the differences of the duplicate analyses within the 42 samples appeared to be normally distributed. Thus, percentiles of the measurement distribution can be assumed to be distributed as normal.

The data (Table 2) from the collaborative study (3 labs, 5 samples, measured in duplicate) contained possible two outlier results. The 4.91 mg/100g result obtained by the second lab for the first replicate of the third sample is quite different from the other five

results, which range from approximately 8 to 9 mg/100g. Thus, this result was not used in the statistical analysis. In addition, for the fifth sample, the sample standard deviation obtained by the first lab, also appears to be an outlier. This can be seen by examining Table 3, which presents means and standard deviations of the replicate results for a sample. The computed ratio, r_{14}, of the maximum sample variance to the sum of the 14 sample variances (excluding the third sample from the second lab) is 0.723, which has statistical significance of $\alpha = 0.0008$. The estimated standard deviation of repeatability (obtained through an analysis of variance), excluding only the outlier result of 4.91 mg/100g was 0.182 mg/100g. When the results for the fifth sample that were obtained by the first lab are also deleted, the estimated standard deviation of repeatability is 0.100 mg/100g.

For deriving the criteria for excess iron in Product produced by advanced recovery systems that can be labeled meat, the repeatability standard deviation is assumed to be 0.16 mg/100g. This is based on the estimated repeatability standard deviation obtained when deleting the 5 samples with standard deviations greater than or equal to 0.58 mg/100g. Support for the 0.16 mg/100g value is the 0.10 mg/100g estimate of the repeatability standard deviation from the collaborative study when the two outlier results are deleted. Because of the few large differences of duplicate sample results, it is recommended there should be at least duplicate analyses on samples used for compliance purposes.

Figure 1: Plot of within sample standard deviations versus sample mean iron level.

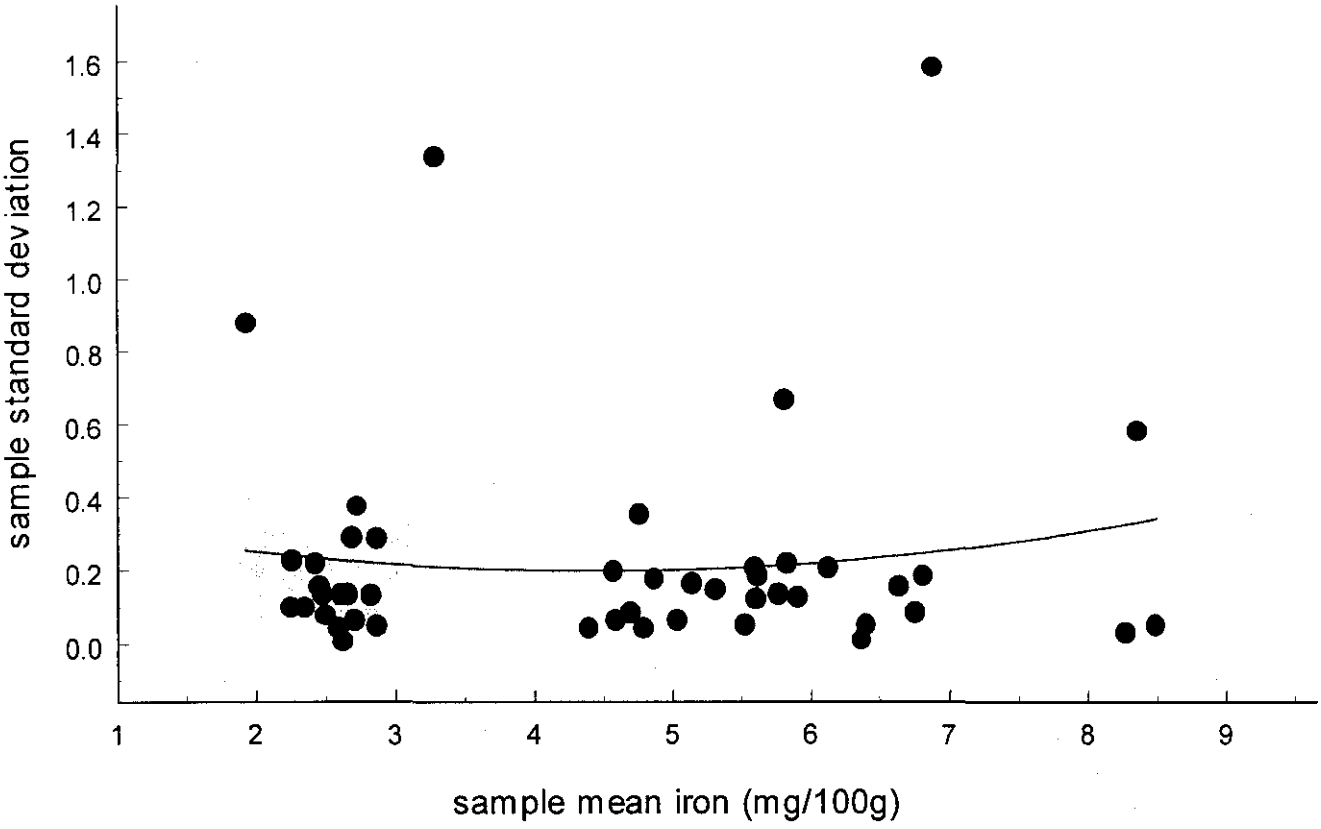

Table 1: Duplicate iron results (mg/100g) from 47 meat samples

sample	replicate 1	2	mean	standard deviation
1	2.33	2.19	2.26	0.10
2	2.63	2.64	2.64	0.01
3	2.29	2.43	2.36	0.10
4	2.35	2.57	2.46	0.16
5	2.58	2.27	2.43	0.22
6	2.90	2.49	2.70	0.29
7	4.24	2.35	3.30	1.34
8	2.63	2.57	2.60	0.04
9	2.47	3.00	2.74	0.37
10	2.84	2.91	2.88	0.05
11	2.67	3.08	2.88	0.29
12	2.74	2.93	2.84	0.13
13	2.58	2.39	2.49	0.13
14	2.72	2.53	2.63	0.13
15	2.67	2.76	2.72	0.06
16	2.56	2.45	2.51	0.08
17	6.95	6.69	6.82	0.18
18	4.64	4.76	4.70	0.08
19	4.52	5.02	4.77	0.35
20	4.83	4.77	4.80	0.04
21	4.74	4.99	4.87	0.18
22	4.37	4.43	4.40	0.04
23	5.82	6.00	5.91	0.13
24	5.42	5.21	5.32	0.15
25	4.55	4.64	4.60	0.06
26	5.67	5.98	5.83	0.22
27	5.56	5.49	5.53	0.05
28	5.52	5.69	5.61	0.12
29	6.38	6.37	6.38	0.01
30	6.70	6.82	6.76	0.08
31	5.03	5.26	5.15	0.16
32	5.68	5.87	5.78	0.13
33	6.44	6.37	6.41	0.05
34	8.30	8.26	8.28	0.03
35	8.46	8.53	8.50	0.05
36	7.95	8.77	8.36	0.58
37	4.71	4.43	4.57	0.20
38	6.53	6.75	6.64	0.16
39	6.29	5.35	5.82	0.66
40	5.45	5.74	5.60	0.21
41	5.48	5.74	5.61	0.18
42	5.00	5.09	5.05	0.06
43	5.77	8.01	6.89	1.58
44	6.27	5.98	6.13	0.21
45	2.10	2.42	2.26	0.23
46	2.76	2.57	2.67	0.13
47	2.55	1.31	1.93	0.88

Table 2: Results from Collaborative study; data provided by ARS

lab	replication	Sample number 1	2	3	4	5
1	1	5.68	6.44	8.30	8.46	8.77
1	2	5.87	6.37	8.26	8.53	7.95
2	1	4.96	5.92	4.91	7.41	7.90
2	2	4.73	5.96	8.00	7.25	8.05
3	1	5.81	5.21	8.99	8.77	8.88
3	2	5.79	5.53	9.03	8.73	8.84

Table 3: Means and standard deviations for samples

	Laboratory						pool	
	1		2		3			
SAMPLE	MEAN	STD	MEAN	STD	MEAN	STD	MEAN	STD
1	5.775	0.134	4.845	0.163	5.800	0.014	5.473	0.122
2	6.405	0.049	5.940	0.028	5.370	0.226	5.905	0.135
3	8.280	0.028	8.000	.	9.010	0.028	8.516	0.028
4	8.495	0.049	7.330	0.113	8.750	0.028	8.192	0.073
5	8.360	0.580	7.975	0.106	8.860	0.028	8.398	0.341